

pSELECT-zeo-GFP::Bsr

A plasmid encoding a CpG-free GFP-Blasticidin resistance fusion gene

Catalog code: psetz-zgfpbsr

For research use only

Version 20K30-MM

PRODUCT INFORMATION

Content:

- 20 µg of pSELECT-zeo-GFP::Bsr plasmid provided as lyophilized DNA.

- 1 ml of Zeocin™ (100 mg/ml)

Storage and Stability:

Product is shipped at room temperature. Lyophilized DNA should be resuspended upon receipt and stored at -20°C. Lyophilized DNA is stable for 3 months at -20°C. Resuspended DNA is stable more than one year at -20°C.

Store Zeocin™ at 4 °C or at -20 °C. The expiry date is specified on the product label.

Quality control:

Plasmid construct has been confirmed by restriction analysis and sequencing. Plasmid DNA was purified by ion exchange chromatography and lyophilized.

GENERAL PRODUCT USE

pSelect-zeo plasmids contain genes that have been chemically synthesized. The DNA sequence of these genes was modified by optimizing the codon usage, reducing or eliminating the CpG motifs and avoiding secondary DNA structures without changing the amino acid sequence of the wild type proteins.

pSelect-zeo plasmids may be used:

To subclone the synthetic gene into another vector. To facilitate subcloning, the GFP::Bsr gene is flanked by two unique restriction sites: Nco I at the 5' end that encompasses the Start codon, and Nhe I at the 3' end.

As a gene reporter plasmid. pSelect-zeo is a mammalian expression plasmid selectable in *E. coli* and mammalian cells with Zeocin™, as the *Sh ble* gene in the second expression cassette is driven by the eukaryote CMV enhancer/promoter in tandem with the bacterial EM7 promoter.

PLASMID FEATURES

First expression cassette

• **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1alpha (EF-1α) core promoter¹ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat². The EF-1α promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1α core promoter to enhance stability of RNA.

• **GFP::Bsr CpG-free:** InvivoGen has engineered a fusion gene between the red-shifted variant of the jellyfish GFP gene that encodes a green fluorescent protein and the *Bsr* gene conferring blasticidin S resistance. Both genes have been modified and contain no CpG motifs, whereas their wildtype counterparts contain 60 and 50 CpG motifs respectively. This GFP::Bsr fusion protein absorbs blue light (major peak at 480 nm) and emits green light (major peak at 505 nm).

• **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA³.

• **ori:** a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

Second expression cassette

• **CMV enh/prom:** The human cytomegalovirus immediate-early gene 1 promoter/enhancer was originally isolated from the Towne strain and was found to be stronger than any other viral promoters.

• **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.

• **Zeo:** Resistance to Zeocin™ is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*. The *Sh ble* gene is driven by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and *E. coli*.

• **βGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁴.

1. Kim, D.W. *et al.* (1990). *Gene* 2: 217-223.

2. Takebe, Y. *et al.* (1988). *Mol. Cell Biol.* 1: 466-472.

3. Carswell, S. & Alwine, J.C. (1989). *Mol. Cell Biol.* 10: 4248-4258.

4. Yu J & Russell JE. (2001). *Mol Cell Biol*, 21(17):5879-88.

METHODS

Plasmid resuspension

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile H₂O. Store resuspended plasmid at -20 °C.

Plasmid amplification and cloning

Plasmid amplification and cloning can be performed in *E. coli* GT116 other commonly used laboratory *E. coli* strains, such as DH5α.

Zeocin™ usage

This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

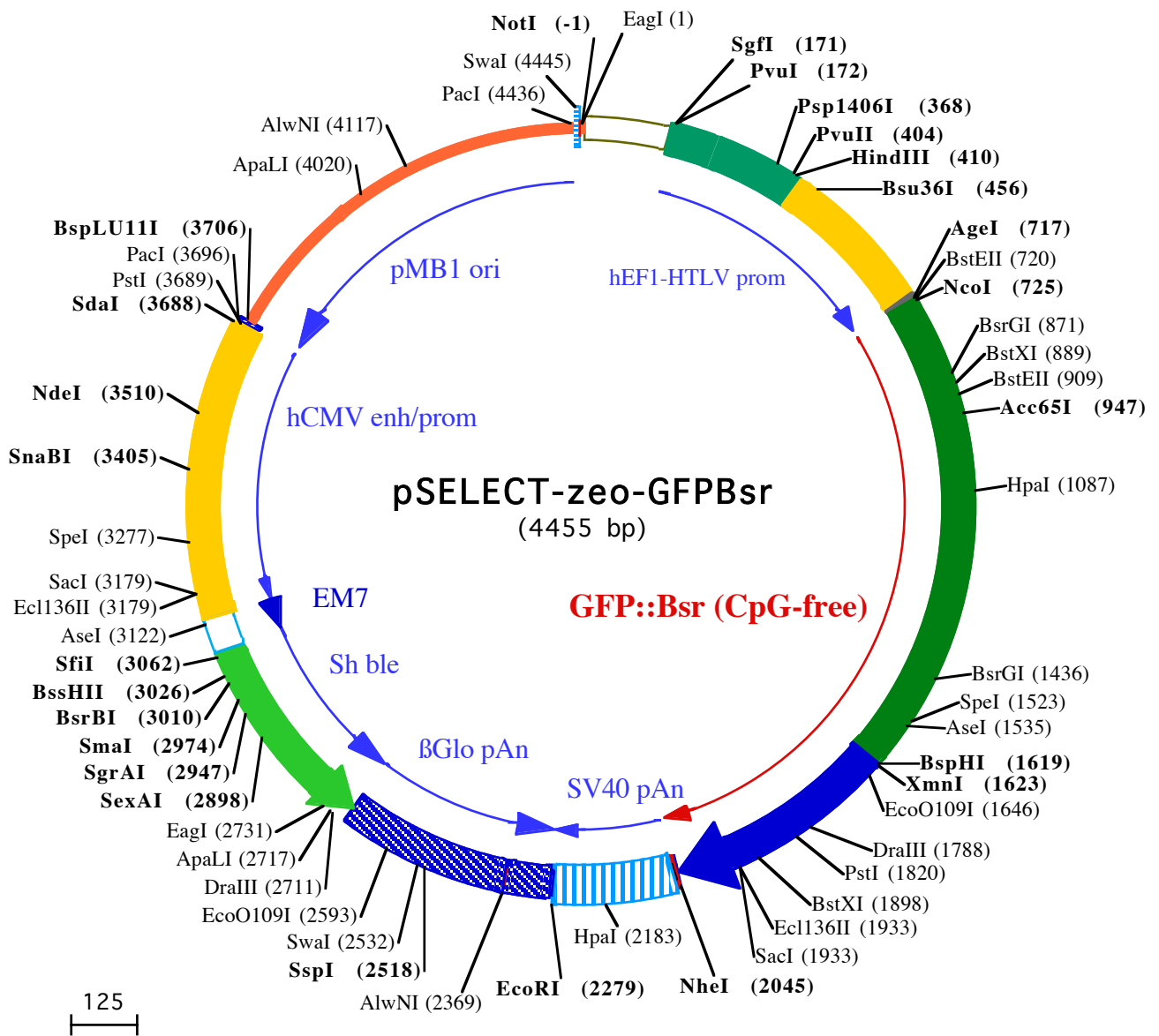
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EagI (1)
NotI (-1)
1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACA

PvuII (172)
SgfI (171)
101 AAACAACTAGCAAAATAGGCTGTCCCGAGTCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTCCCGTCACTGGGCA

201 GAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGTCCGCAATTGAACGGTGCTAGAGAAGTGGCGGGGTAAACTGGGAAAGTGATG

Psp1406I (368)
301 TCGTGACTGGCTCCGCTTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCGGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAG

HindIII (410)
PvuII (404) Bsu36I (456)
401 AACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTACGCGCCCGCCCTACCTGAGGCCCATCCACGCCGTTGAGTCGCGTTCTGCCGCT

501 CCGCCTGTGGTGCCTCTGAAGTCCGCTCCGCTAGGTAAGTTAAAGCTCAGGTCGAGACCGGGCTTTGTCCGGCGCTCCCTTGGAGCTACCTA

601 GACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTCAACTCTACGCTTTTGTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACC

NeoI (725)
BstEII (720)
AgeI (717)
701 GCGCCTACCTGAGATCACCGgtcaCCATGGTTCTAAGGGAGAAGAACTCTTACTGGTGTGTCCCAATTCTGGTTGAGCTGGATGGTGTGAATG

1 M V S K G E E L F T G V V P I L V E L D G D V N

BsrGI (871) BstXI (889)
801 GCCACAAATCTGTGTCTGGTGAAGGTGAAGGAGATGCAACTTATGAAAGCTGACTCTGAAGTTCATTTGTACAACAGGAAAGCTGCCAGTGCCTTG

25 G H K F S V S G E G E G D A T Y G K L T L K F I C T T G K L P V P W

BstEII (909) Acc65I (947)
901 GCCAACTCTGGTGACCACCTGACTTATGGTGTCAATGTTTCAGCAGGTACCCCTGACCACATGAAGCAGCATGACTTCTTTAAATCTGCAATGCCAGAA

58 P T L V T T L T Y G V Q C F S R Y P D H M K Q H D F F K S A M P E

HpaI (1087)
1001 GGTATGTTCAAGGAGGACAATCTTCTTTAAGGATGATGAAATTATAAGACAAGGCGAGAAGTGAAGTTGAAGGTGATACACTGGTTAACAGAAATTG

92 G Y V Q E R T I F F K D D G N Y K T R A E V K F E G D T L V N R I

1101 AGCTGAAAGCATTGATTTAAAGGAAGTGAAGAACTTCTGGTCCACAGCTGGAGTACAACATAATTTCTCACAATGTTTACATTATGGCAGATAAGCA

125 E L K G I D F K E D G N I L G H K L E Y N Y N S H N V Y I M A D K Q

1201 GAGGAATGGAATTAAGGCTAATTTCAAGATTAGACACAACATTGAGGATGGATCTGTCCAAGTCCAGACATTACCAGCAGAACCCTTATTGGTGT

158 R N G I K A N F K I R H N I E D G S V Q L A D H Y Q Q N T P I G D

1301 GGCCAGTCTCTCCAGATAATCACTATCTCAGCACTCAATCTGTCTGTCCAAAGACCTAATGAGAAAAGACACACATGGTCTCTGGAGTTTG

192 G P V L P D N H Y L S T Q S A L S K D P N E K R D H M V L L E F

BsrGI (1436)
1401 TGACGACAGCAGGAATTAAGTCTGGGAATGGATGAGCTGTACAAGGgtaagtcactgactgtctatgcctgggaaagggtgggcaggagatggggcagtg

225 V T A A G I T L G M D E L Y K G K S L T V Y A W E R V G R R W G S A

SpeI (1523) AseI (1535)
1501 aggaaaagtggcactatgaaccACTAGTTTGAACAATTAATCATAAGCATAGTATAATACAACCTACTATAGcaattgtactaaccttcttctcttct

258 G K V A L • T H • F D N • S • A • Y N T T H Y S N C T N L L L F

XmnI (1623)
BspHI (1619) EcoO109I (1646)
1599 ctctcctgacagGAGGAGCCATCATGAAGACCTTCAACATCTCTCAGCAGGACCTTGAGCTGGTGAAGTTGCCACTGAGAAAATCACCATGCTCTATG

291 P L L T G G A I M K T F N I S Q Q D L E L V E V A T E K I T M L Y

DraIII (1788)
1698 AGGACAACAAGCACCATGTTGGTCTGCCATCAGGACCAAGACAGGAGAAATCATCTCTGTGTCCACATTGAAGCCTACATTGGCAGGGTCACTGTGTG

324 E D N K H H V G A A I R T K T G E I I S A V H I E A Y I G R V T V C

PstI (1820)
1798 TGACAGAGCCATTGCCATTGGTCTGCAGTCTCCAATGGGCGAGAAGGACTTTGACACCATTGTGGCTGTGAGGCACCCCTACAGTGTGAGGTGGACAGG

357 A E A I A I G S A V S N G Q K D F D T I V A V R H P Y S D E V D R

SacI (1933)
Ecl136II (1933)
1898 TCCATCAGAGTGGTGTCCCTGTGGCATGTGCAGGGAGCTCATCTCAGACTATGCCCTGATTGCTTTGTTCTGATTGAGATGAATGGCAAGCTGGTCA

391 S I R V V S P C G M C R E L I S D Y A P D C F V L I E M N G K L V

NheI (2045)
1998 AGACAACATTGAGGAGCTGATCCACTGAATACACCAGAACTAAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTGGACAACACCAA

424 K T T I E E L I P L K Y T R N

HpaI (2183)
2098 CTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAGTTAACAAACAA

EcoRI (2279)
2198 TTGCATTCATTTTATGTTTCAGGTTCCAGGGGAGGTGTGGGAGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATCTAAAATACAGCA

AlwNI (2369)
2298 TAGCAAACTTTAACCTCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGCTGTGGCAATGTGCATTAGCT

2398 GTTTCAGCCTCACCTCTTTCATGGAGTTAAGATATAGTGATTTTCCCAAGGTTTGAAGTACTCTTCTTCTTTATGTTTTAAATGCACTGACCT

SspI (2518) SwaI (2532) EcoO109I (2593)
2498 CCCACATTCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGC

2598 CCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGAACCTTTAATAGAATTGGACAGCAAGAAGCGAGCTTCTAGCTTATCCTCAG

127 • G •

ApaLI (2717)
DraIII (2711) EagI (2731)
2698 TCCTGCTCCTCTGCCACAAAGTGACGAGTTGCCGGCCGGTCCGCGAGGGCGAACTCCCGCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCC

123 D Q E E A V F H V C N G A P D R L A F E R G W P Q E G I E T M A P G

2798 CGGAGGCGTCCCGAAGTTCGTGGACACGACTCCGACCACTCGGCGTACAGCTGCTCCAGGCGCGCACCCACACCCAGGCCAGGGTGTTCGGCCAC

90 S A D R F N T S V V E S W E A Y L E D L G R V W V W A L T N D P V

SexAI (2898) SgrAI (2947) SmaI (2974)
2898 CACTGGTCTTGGACCGCGTGTGAACAGGGTACGCTGCTCCCGACCAACCCGGCGAAGTCTCTCCACGAAGTCCCGGAGAACCCGAGCCGGCTG

574 V Q D Q V A S I F L T V D D R V V G A F D D E V F D R S F G L R D

2998 **BsrBI (3010)** **BssHII (3026)** **SfiI (3062)**
 GTCCAGAACTCGACCGCTCCGGCGACGTCCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTGGCCATGATGGCCCTCTATAGTGAGTCGTATTAT
 234 T W F E V A G A V D R A T L V P V A S T L K A M

3098 **AseI (3122)** **SacI (3179)** **Ecl136II (3179)**
 ACTATGCCGATATACTATGCCGATGATTAATTGTCAAACACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCACATAAACGAGCTCTGCTTATATAGA

3198 **SpeI (3277)**
 CCTCCCACCGTACACGCCTACCGCCATTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAAGTCCCGTTGATTACTAGTCAAAAACAACTCCC

3298
 ATTGACGTCAATGGGGTGGAGACTTGGAAATCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTAAGTCCAAAACCGCATCATCATGGTAATAGCG

3398 **SnaBI (3405)**
 ATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCATAAGGTGATGTAAGTGGGCATAATGCCAGGGGGCCATTACCGTCATTGACGTCAATAG

3498 **NdeI (3510)**
 GGGGCGTACTTGGCATATGATACACTTGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCATTGACGTCAATGGAAAGTCCCTATTGGCGT

3598 **PstI (3689)** **SdaI (3688)** **PacI (3696)**
 TACTATGGGAACATACGTCATTATTGACGTCAATGGGGCGGGTCTGTTGGGCGGTGAGCCAGGCGGGCCATTACCGTAAAGTTATGTAACGCCTGCAGGT

3698 **BspLU11I (3706)**
 TAATTAAGAAACATGTGAGCAAAAGGCCAGCAAAAGCCAGGAACCGTAAAAAGGCCGCTTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCA

3798
 TCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGACTATAAAGATACAGGCGTTTCCCGTGGAAAGCTCCCTCGTGCCTCTCCTGTT

3898
 CCGACCTGCCGTTACCGGATACCTGTCCGCTTTTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGG

3998 **ApaLI (4020)**
 TCGTTCGCTCCAAGCTGGGCTGTGTGCACGAAACCCCGTTCAGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGTAGTCCAAACCGGTAAGACA

4098 **AlwNI (4117)**
 CGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGATTCTTGAAGTGGTGGCCTAACTACGGC

4198
 TACTACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCCGCTG

4298
 GTAGCGGTGGTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTG

4398 **PacI (4436)** **SwaI (4445)**
 GAACGAAAACACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAAACATTTAAATCA